Remarks

Reconsideration of this Application is respectfully requested.

Claims 71-73, 75-78, 80-82 and 99 are pending in the application, with claims 71 and 80 being the independent claims. Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 102

The Examiner maintained the rejection of claims 71-73, 75-78, 80-82 and 99 under 35 U.S.C. §102(b) as allegedly being anticipated by Tomassini (Ph.D. Dissertation, 1986) [hereinafter "Tomassini thesis"], Tomassini *et al.* (*J. Virol. 58*:290-295 (1986)) [hereinafter "Tomassini article"], and Colonno *et al.* (Virus Attachment and Entry into Cells, Proceedings of an ASM Conference held in Philadelphia, PA, April 10-13, 1985) [hereinafter "Colonno article"]. (Paper No. 30, at pages 2-3.) According to the Examiner,

> no more of the reference is required than that is sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced rhinovirus receptor.

Id. at page 3. Applicants respectfully disagree.

For a 102(b) rejection to be proper, each and every element of the claims must be found in the cited reference. Also, "the identical invention must be shown in as complete detail as is contained in the . . . claim." M.P.E.P. § 2131 at 2100-69 (2001). Furthermore, to anticipate, the reference must also enable one of skill in the art to make and use the claimed invention. *In re Donohue*, 766 F.2d 531, 533, 226 U.S.P.Q. 619, 621 (Fed. Cir. 1985).

As Applicants previously indicated, the isolation and purification of an active form of a membrane-associated protein, such as ICAM-1, is highly dependent on the purification procedure used. Integral membrane proteins require high concentrations of detergent for solubilization and generally complete solubilization is needed to release them. Integral membrane proteins are normally neither soluble nor stable in the absence of detergent. *Current Protocols in Protein Science*, Strategies for Protein Purification, Unit 1.2 at 1.2.2 (1995). Thus, the purification of membrane-associated proteins, such as ICAM-1, is not a trivial procedure. Moreover, there is no guarantee that any purification procedure will yield a *functional* form of ICAM-1. In fact, the authors of the Tomassini thesis and article indicate that using their purification procedure, they are *unable* to isolate a functional 90-kDa receptor protein (ICAM-1) capable of binding virus (Tomassini thesis at 116, line 22, to 117, line 1; and Tomassini article at 295, col. 1, lines 20-25.). Thus, the purification procedure taught by the Tomassini thesis and article renders the isolated HRRP preparation nonfunctional.

In addition, there are distinct differences between Tomassini's purification procedure and Applicants' procedure. One critical difference is the type of detergent used in the purification procedure. The detergent used by Tomassini *et al.* in their HRRP purification procedure is sodium deoxycholate (*see* page 39 of the Tomassini thesis and page 291 of the Tomassini article). Sodium deoxycholate is an ionic detergent. In contrast, the detergent used by Applicants in their ICAM-1 purification procedure is Triton X-100 (*see* page 62 of the specification). Triton X-100 is a non-ionic detergent. Ionic and non-ionic detergents can differ in their ability to denature proteins. *See* attached copy of *Current Protocols in Protein Science*, Commonly Used Detergents, Appendix 1B at A.1B.1 (1998), stating that "[i]onic detergents are very good solubilizing agents, but they tend to denature proteins by

destroying native three-dimensional structures." *Id.* at A.1B.2. Since the purification procedure used by Tomassini, employing an ionic detergent, renders their HRRP preparation nonfunctional, it is likely that their HRRP preparation was denatured to such an extent that it was not capable of binding to virus. In contrast, Applicants' HRRP preparation was purified using a non-ionic detergent, and was functional and capable of binding to virus.

Based on the teachings of the Tomassini thesis and the Tomassini article, and the fact that the binding sites for LFA-1 and HRV overlap, one of ordinary skill in the art would have no reason to believe that purified HRRP would bind to LFA-1, Mac-1, or p150,95. Therefore, the Tomassini thesis and the Tomassini article do not teach the isolation of HRRP in active form. No other art has been cited by the Examiner to establish the purification of HRRP in an active form. Thus, regardless of whether or not numerous purification procedures were available at the time the invention was made, the Tomassini thesis and article and Colonno article cited by the Examiner do not teach the isolation of a *functional* form of ICAM-1.

Moreover, Colonno *et al.* show "a predominant protein band migrating with an apparent molecular weight of 90,000 (J. E. Tomassini and R. J. Colonno, submitted for publication)." (Colonno *et al.*, page 113, lines 29-31.) However, "[f]urther analysis of this candidate receptor protein is in progress." *Id.* at lines 34-35. Thus, Colonno *et al.*, mentions the Tomassini article discussed above for further analysis of the receptor protein. As discussed above, neither the Tomassini article nor the Tomassini thesis teach the isolation of an active form of ICAM-1, capable of binding to HRV, LFA-1, Mac-1 or p150,95.

In contrast, Applicants' purification procedure taught in the specification enables isolation of a functional HRRP receptor (ICAM-1), capable of binding to LFA-1, Mac-1 or p150,95. Since it is generally known in the art that activity of an isolated and purified

protein depends primarily on the purification procedure used, the claimed functional limitations *cannot* be inherent properties of the referenced rhinovirus receptor. Moreover, the art cited by the Examiner is proof that protein purification procedures may or may not result in the isolation of a functional protein. Since the Tomassini thesis and article and the Colonno article do not teach the isolation of ICAM-1 in active form as presently claimed, this rejection should be withdrawn.

The Examiner further stated that "[t]he burden is on the applicant to establish a patentable distinction between the claimed and referenced products." (Paper No. 30, at page 3.)

Applicants contend that they have met this burden. As discussed *supra*, the art cited by the Examiner does not teach the isolation and purification of ICAM-1 in active form as presently claimed.

Rejections under 35 U.S.C. § 103

The Examiner maintained the rejection of claims 71-73, 75-78, 80-81 and 99 under 35 U.S.C. §103(a) as allegedly being unpatentable over the Tomassini thesis, the Tomassini article and/or the Colonno article. (Paper No. 30, at page 3.) According to the Examiner,

> [w]hile it is acknowledged that isolation and purification of an active form of a membrane-associated protein is dependent on the purification procedure used; it was certainly within the purview of the ordinary artisan to isolate and purify functional forms of a known [sic] at the time the invention was made, given the arsenal of isolation and purification methods known and practiced at the time the invention was made.

Id. at page 5. Applicants respectfully disagree.

To establish a *prima facie* case of obviousness under 35 U.S.C. § 103, the Examiner must show that the prior art suggested to those of ordinary skill in the art that they should make the claimed composition or device, and that the invention could be obtained with a reasonable expectation of success. *See In re Vaeck*, 20 U.S.P.Q.2d 1438, 1443 (Fed. Cir. 1991).

As Applicants previously indicated, the isolation and purification of an active form of a membrane-associated protein, such as ICAM-1, is highly dependent on the purification procedure used. Integral membrane proteins require high concentrations of detergent for solubilization and generally complete solubilization is needed to release them. Integral membrane proteins are normally neither soluble nor stable in the absence of detergent. *Current Protocols in Protein Science*, Strategies for Protein Purification, Unit 1.2 at 1.2.2 (1995). Thus, the purification of membrane-associated proteins, such as ICAM-1, is not a trivial procedure. Moreover, there is no guarantee that any purification procedure will yield a *functional* form of ICAM-1. In fact, the authors of the Tomassini thesis and article indicate that using their purification procedure, they are *unable* to isolate a functional 90-kDa receptor protein (ICAM-1) capable of binding virus (Tomassini thesis at 116, line 22, to 117, line 1; and Tomassini article at 295, col. 1, lines 20-25.). Thus, regardless of whether or not numerous purification procedures were available at the time the invention was made, from the teachings of the references, one skilled in the art would have no reasonable expectation of success in producing an ICAM-1 preparation capable of binding to HRV, LFA-1, Mac-1 or p150,95.

The Examiner alleged that "applicant has failed to rebut prima facie showing of inherency or obviousness absent objective evidence such as_side-by-side testing that would

address the ability of the prior art HRV receptors ability to bind LFA-1/Mac-1/p150,95." (Paper No. 30, at page 6.) Applicants respectfully disagree.

Applicants contend that they have successfully rebutted the Examiner's showing of inherency or obviousness. As Applicants indicated *supra* and have indicated *infra*, it is not inherent at the time the invention was made that the HRV receptor identified in the references had the ability to bind virus, since the authors of the Tomassini thesis and the Tomassini article indicated that using their purification procedure, they were unable to isolate a 90-kDa receptor protein capable of binding virus. Since it is generally known in the art that activity of an isolated and purified protein depends primarily on the purification procedure used, the claimed functional limitations *cannot* be inherent properties of the referenced rhinovirus receptor. Thus, no side-by-side testing is necessary, since the references cited by the Examiner teach the isolation of an *inactive* form of HRRP *incapable* of binding to rhinovirus.

The Examiner reiterated rejections of record, stating that "[e]ven if there is an indication that there may be reduced binding of a particular radiolabeled HRV receptor preparation reduced binding to HRV [sic]; it maintained the ability to bind." (Paper No. 30, at page 6.)

Applicants respectfully disagree. There was no indication of reduced binding of the radiolabeled HRV receptor preparation to HRV; there was *no* binding. This indicates that the radiolabeled HRV receptor preparation is inactive and incapable of binding virus.

The Examiner further stated that "[i]t is clear that the Tomassini thesis as well as the other references clearly teach that the HRV receptor is indeed the receptor for rhinovirus, that the HRV receptor is bound by antibodies that block HRV attachment or binding, and

that the HRV receptor can be used as an immunogen to produce an antibody that blocks HRV attachment and binding." (Paper No. 30, at page 6.)

Applicants contend that it is not at all clear from the Tomassini thesis and the references that the HRV receptor is the receptor for rhinovirus since the thesis does not teach the isolation and purification of an active form of ICAM-1 capable of binding to HRV, LFA-1, Mac-1 or p150,95. In addition, the thesis and references do not actually show that the HRV receptor preparation is bound by antibodies that block HRV attachment or binding. Instead, the thesis and references show, for example, that addition of increasing amounts of receptor antiserum corresponded to an increased inhibition of ^{35}S-labeled rhinovirus binding to HeLa membranes. (Tomassini thesis at page 65, lines 4-8.)

Furthermore, Dr. Rothlein, in his Rule 132 Declaration submitted on October 24, 2000, discussed the fact that the cDNA clones disclosed in the Tomassini thesis would not express ICAM-1, and that the clones represent a cloning artifact or a fortuitous cross-reactivity of the anti-HRV-receptor antibody with another anti-ICAM-1 protein.

As further evidence that the Tomassini clones do not comprise the actual ICAM-1 gene, a subsequent article published by the author of the thesis, Tomassini *et al.*, *Proc. Natl. Acad. Sci. 86*:4907-4911 (1989) (not prior art), teaches the cloning of the ICAM-1 gene. (*See* Exhibit H submitted with the Rule 132 Declaration.) To obtain the cloned gene, Tomassini *et al.* used a different cDNA library and different clones than the library and clones described in the thesis. If the clones described in the thesis actually contained the ICAM-1 gene, it would not have been necessary to clone the ICAM-1 gene from another source. Finally, the monoclonal antibody directed against the HRV receptor (ICAM-1) did not recognize the protein expressed from clone 4A, showing that the portion of ICAM-1

recognized by the antibody was not expressed in its native state. (Tomassini thesis at 85, lines 16-20.)

The Examiner also stated that "[e]ither it was inherent or expected at the time the invention was made that the HRV receptor identified and characterized by the references had the ability to bind virus and, in turn, would have either the inherent or expected properties of binding LFA-1/Mac-1/p150,95." (Paper No. 30, at page 6.)

Applicants contend that it was neither inherent nor expected at the time the invention was made that the HRV receptor identified in the references had the ability to bind virus, since the authors of the Tomassini thesis and the Tomassini article indicated that using their purification procedure, they were unable to isolate a 90-kDa receptor protein capable of binding virus. Since it is generally known in the art that activity of an isolated and purified protein depends primarily on the purification procedure used, the claimed functional limitations *cannot* be inherent properties of the referenced rhinovirus receptor. Moreover, one with ordinary skill in the art would have no reason to expect that their HRRP purification procedure would yield a functional form of HRRP capable of binding to LFA-1, Mac-1, or p150,95, since the references cited by the Examiner teach the isolation of an *inactive* form of HRRP *incapable* of binding to rhinovirus.

In contrast, Applicants' claimed invention relates to an isolated and purified ICAM-1 preparation, capable of binding to LFA-1, Mac-1 or p150,95. Since the Tomassini thesis and the article do not teach the isolation of HRRP in active form as presently claimed, withdrawal of this rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

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CORE 6 (S23)

Commonly Used Detergents

Detergents are polar lipids that are soluble in water. The presence of both a hydrophobic and hydrophilic portion makes these compounds very useful for lysis of lipid membranes, solubilization of antigens, and washing of immune complexes.

TYPES OF DETERGENTS

A large variety of detergents are available (Helenius et al., 1979). For biochemical studies, they are usually categorized according to the type of hydrophilic group—anionic, cationic, amphoteric, or nonionic. Tables A.1B.1 and A.1B.2 list commonly used members of each type. In general, nonionic and amphoteric detergents are less denaturing for proteins than ionic detergents. Sodium cholate and sodium deoxycholate are the least denaturing of the commonly used ionic detergents.

Two properties of detergents are important in their consideration for biological studies: the critical micelle concentration (CMC) and the micelle molecular weight (Table A.1B.1). The CMC is the concentration at which monomers of detergent molecules combine to form micelles; each detergent micelle has a characteristic micelle molecular weight. Detergents with a high micelle molecular weight such as nonionic detergents are difficult to remove from samples by dialysis. The CMC and the micelle molecular weight will vary depending on the buffer, salt concentration, pH, and temperature. In general, adding salt will lower the CMC and raise the micelle size.

Table A.1B.1 Physical Properties of Commonly Used Detergents[a,b]

Detergent	mp (°C)	Molecular weight (Da)		CMC	
		Monomer	Micelle	% (w/v)	M
Anionic					
SDS	206	288	18,000	0.23	8.0×10^{-3}
Cholate	201	430	4,300	0.60	1.4×10^{-2}
Deoxycholate	175	432	4,200	0.21	5.0×10^{-3}
Cationic					
C_{16}TAB	230	365	62,000	0.04	1.0×10^{-3}
Amphoteric					
LysoPC	—	495	92,000	0.0004	7.0×10^{-6}
CHAPS	157	615	6,150	0.49	1.4×10^{-3}
Zwittergent 3-14	—[a]	364	30,000	0.011	3.0×10^{-4}
Nonionic					
Octyl glucoside	105	292	8,000	0.73	2.3×10^{-2}
Digitonin	235	1,229	70,000	—	—
$C_{12}E_8$	—	542	65,000	0.005	8.7×10^{-5}
Lubrol PX	—	582	64,000	0.006	1.0×10^{-4}
Triton X-100	—	650	90,000	0.021	3.0×10^{-4}
Nonidet P-40	—	603	90,000	0.017	3.0×10^{-4}
Tween 80	—	1,310	76,000	0.002	1.2×10^{-5}

[a] Reprinted with permission from IRL Press (see Jones et al., 1987).

[b] Abbreviations: C_{16}TAB, hexadecyl trimethylammonium bromide; CMC, critical micelle concentration; LysoPC, lysophosphatidylcholine; mp, melting point; SDS, sodium dodecyl sulfate.

Contributed by John E. Coligan

Current Protocols in Protein Science (1998) A.1B.1-A.1B.3

Table A.1B.2 [redacted]mical Properties of Commonly Used Detergen[redacted]

Property	Ionic detergents							Nonionic detergents						
	SDS	CHO	DOC	C_{16}	LYS	CHA	ZWI	OGL	DIG	C_{12}	LUB	TNX	NP-40	T80
Strongly denaturing[c]	+	–	–	+	+/–	–	+/–	–	–	–	–	–	–	–
Dialyzable	+	+	+	+	–	+	+/–	+	–	–	–	–	–	–
Ion exchangeable[d]	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Complexes ions	+	+	+	–	–	–	–	–	–	+/–	+/–	+/–	+/–	+/–
Strong A_{280}	–	–	–	–	–	–	–	–	–	–	–	+	+	–
Assay interference	–	–	–	–	–	–	–	–	–	–	+/–	+/–	+/–	+/–
Cold precipitates	+	–	+	+	–	–	–	–	–	–	–	–	–	–
High cost	–	–	–	–	+	+	+	+	+	+	–	–	–	–
Availability	+	+	+	+	+	+	+/–	+	+	+/–	+	+	+	+
Toxicity	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ease of purification	+	+	+	+	+/–	+	+	–	+	–	–	–	–	–
Radiolabeled	+	+	+	–	+	–	–	+	–	+	+	+	+	+
Defined composition	+	+	+	+	+	+	+	+		–	–	–	–	–
Auto-oxidation	–	–	–	–	–	–	–	–		+	+	+	+	+

[a]Adapted from IRL Press (see Jones et al., 1987).

[b]Abbreviations: C_{12}, $C_{12}E_8$; C_{16}, hexadecyl trimethylammonium bromide; CHA, CHAPS; CHO, cholate; DIG, digitonin; DOC, deoxycholate; LUB, lubrol PX; LYS, lysophosphatidylcholine; NP-40, Nonidet 40; OGL, octyl glucoside; SDS, sodium dodecyl sulfate; T80, Tween 80; TNX, Triton X–100; ZWI, Zwittergent 3-14.

[c] Denaturing refers to disruption of secondary and tertiary protein structure.

[d]Ionic detergents are unsuitable for ion-exchange chromatography (*UNIT 8.2*).

CHOICE OF DETERGENTS

Ionic detergents are very good solubilizing agents, but they tend to denature proteins by destroying native three-dimensional structures. This denaturing ability is useful for SDS-PAGE (*UNIT 10.1*), but is detrimental where native structure is important, as when functional activities must be retained (antibody activity is usually retained in <0.1% SDS). Nonionic and mildly ionic detergents are less denaturing and can often be used to solubilize membrane proteins while retaining protein-protein interactions. The following detergent properties are detrimental in certain procedures:

1. Phenol-containing detergents (e.g. Triton X-100 and NP-40) have a high absorbance at 280 nm and hence interfere with protein monitoring during chromatography (most ionic detergents do not absorb at 280 nm; Brij- and Lubrol-series detergents are nonionic detergents that do not have substantial absorbance at 280 nm). Phenol-containing detergents also induce precipitation in the Folin protein assay (but they can be used with the Bradford protein assay; *UNIT 3.4*). Finally, they are readily iodinated and so should not be present during radioiodination.

2. Many detergents have a very high micelle molecular weight (Table A.1B.1) which makes their use in gel filtration impossible since protein sizes are insignificant relative to the micelle size. In addition, these cannot be readily removed by dialysis.

3. Sodium cholate and sodium deoxycholate are insoluble below pH 7.5 or above an ionic strength of 0.1%. SDS will often crystallize below 20°C.

4. Ionic detergents interfere with nondenaturing electrophoresis (*UNIT 10.3*) and isoelectric focusing (*UNIT 10.2*).

Detergents can be removed or exchanged for other detergents by a variety of procedures (Harlow and Lane, 1988; Furth et al., 1984; Hjelmeland, 1979). Ionic and amphoteric detergents can usually be removed by dialysis *UNIT 4.4*. Pierce makes Extracti-Gel D for removing a variety of detergents from protein solutions (*Pierce Immunotechnology Catalog* and *Handbook on Protein Modification*).

LITERATURE CITED

Furth, A.J., Bolton, H., Potter, J., and Priddle, J.D. 1984. Separating detergents from proteins. *Methods Enzymol.* 104:318-328.

Harlow, E. and Lane, D. 1988. Detergents. *In* Antibodies: A Laboratory Manual, pp. 687-689. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Helenius, A., McCaslin, D.R., Fries, E., and Tanford, C. 1979. Properties of detergents. *Methods Enzymol.* 56:734-749.

Hjelmand, L.M. 1979. Removal of detergents from membrane proteins. *Methods Enzymol.* 182:277-282.

Jones, O.T., Earnest, J.P. and McNamee, M.G. 1987. Solubilization and reconstitution of membrane proteins. *In* Biological Membranes: A Practical Approach (J. Findlay and W.H. Evans, eds.), pp. 142-143. IRL Press, Oxford.

KEY REFERENCES

Harlow and Lane, 1988. See above.

Provides properties of commonly used detergents and means of removing them from proteins.

Hjelmeland, L.M. and Chrambach, A. 1984. Solubilization of functional membrane proteins. *Methods Enzymol.* 182:305-318.

Describes properties of detergents and how to use them to solubilize proteins.

Johnstone, A. and Thorpe, R. 1982. Isolation and fractionation of lymphocytes. *In* Immunochemistry in Practice, pp. 94-101. Blackwell Scientific, Oxford.

Provides details in use of detergents to solubilize cells and membranes.

Neugebaur, J.M. 1990. Detergents: An overview. *Methods Enzymol.* 182:239-282.

Provides details on detergent properties and choosing one for a particular application.

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